ANNALS OF SURGERY
Vol. 228, No. 6, 788–796

Warm Ischemic Tolerance in Collapsed Pulmonary Grafts Is Limited to 1 Hour

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Objective
To determine the length of warm ischemic tolerance in pulmonary grafts from non-heart-beating donors.

Summary Background Data
If lungs could be retrieved for transplant after circulatory arrest, the shortage of donors might be significantly alleviated. Great concern, however, exists about the length of tolerable warm ischemia before cold preservation of pulmonary grafts retrieved from such non-heart-beating donors.

Methods
The authors compared the influence of an increasing postmortem interval on graft function in an isolated, room air-ventilated rabbit lung model during blood reperfusion up to 4 hours. Four groups of cadavers (four animals per group) were studied. In group 1, lungs were immediately reperfused. In the other groups, cadavers with lungs deflated were left at room temperature for 1 hour (group 2), 2 hours (group 3), or 4 hours (group 4).

Results
Pulmonary vascular resistance was enhanced in all ischemic groups compared with the control group. An increase was noted with longer postmortem intervals in peak airway pressure and in weight gain. A concomitant decline was observed in the venoarterial oxygen pressure gradient caused by progressive edema formation, as reflected by the wet-to-dry weight ratio at the end of reperfusion.

Conclusions
Warm ischemia resulted in increased pulmonary vascular resistance. Graft function in lungs retrieved 1 hour after death was not significantly worse than in nonischemic lungs. Therefore, 60 minutes of warm ischemia with the lung collapsed may be tolerated before cold storage. Further studies are necessary to investigate whether lungs retrieved from non-heart-beating donors will become a realistic alternative for transplant.

Lung transplantation, like other forms of solid organ transplantation, is limited by a scarcity of good donor organs. It is estimated that fewer than 10% of all available multiorgan donors have lungs suitable for lung transplantation.[1] To alleviate this critical organ shortage, there is a growing interest in increasing the potential donor pool by turning to alternative sources such as the use of lobar or split transplants,[2,3] living-related donors,[4] or organs from circulation-arrested cadavers, so-called non-heart-beating donors (NHBDs). In NHBDs, however, there will always be a certain delay between (unexpected) circulatory arrest and the start of cold *in situ* flush of the organs. This period of inevitable warm ischemia in the NHBD should be kept as short as possible. However, organizing organ retrieval and obtaining family consent for organ donation consumes precious time.

The clinical use of lungs from NHBDs is still anecdotal.[5,6] Nevertheless, transplantation of lungs retrieved from cadavers after cardiac arrest has been investigated in an increasing number of animal transplant experiments during recent years.[7–12] In previous rabbit studies from our laboratory, we have investigated the effect of postmortem

Presented at the Fifth Annual Meeting of the European Surgical Association, Milan, Italy, April 23–24, 1998.
Supported by a grant from the Nationaal Fonds voor Wetenschappelijk onderzoek—Levenslijn 1994, #7.0036.94.
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Accepted for publication July 13, 1998.

Table 1. ANIMAL CHARACTERISTICS IN DIFFERENT STUDY GROUPS*

Group	Animal Weight (g)	Heart-Lung Block Weight† (g)	Ischemic Time (min)	Preparation Time† (min)	Blood Volume (ml)	Perfusate Volume (ml)
1: CONTR	2353 ± 44	40 ± 1	0 ± 0	29 ± 1	338 ± 11	451 ± 15
2: DEFL-1	2727 ± 69‡	43 ± 1	60 ± 0	28 ± 0	324 ± 9	432 ± 12
3: DEFL-2	2575 ± 105	39 ± 1	120 ± 0	28 ± 0	410 ± 15§	547 ± 21§
4: DEFL-4	2466 ± 64	44 ± 2	240 ± 0	28 ± 1	335 ± 4	447 ± 5

* Values are means ± standard error of mean from four experiments.
† Not significant between all groups (analysis of variance with factorial analysis).
‡ $p < 0.05$ DEFL-1 *versus* CONTR (analysis of variance with factorial analysis).
§ $p < 0.01$ DEFL-2 *versus* other groups (analysis of variance with factorial analysis).
CONTR = Control; DEFL-1 = Deflated 1 hour; DEFL-2 = Deflated 2 hours; DEFL-4 = Deflated 4 hours.

cadaver lung inflation, ventilation, and cooling on the catabolism of adenine nucleotides[13] and on pulmonary cell viability.[14,15] We have also examined the effect of external cadaver cooling on pulmonary temperatures[16] at intervals after death. Recently, we have demonstrated the beneficial effect of postmortem alveolar expansion by inflating or ventilating the cadaver lung on subsequent graft function.[17,18] In the clinical setting of uncontrolled NHBD (Maastricht categories I and II[19]), however, the cadaver will be left with lungs collapsed for a certain period before postmortem alveolar expansion can be instituted.

In the present study, using an isolated rabbit lung reperfusion model, we investigated the influence of an increasing postmortem time interval in the nonmanipulated cadaver on subsequent pulmonary graft function.

MATERIALS AND METHODS

Experimental Groups

Sixteen New Zealand white rabbits (mean weight 2530 ± 49 g) were killed and assigned to four groups of four animals per group. In group 1 (control group), both lungs were immediately excised and prepared for isolated reperfusion. In the other groups, cadavers were left at room temperature (approximately 24°C) with lungs deflated and sternal edges reapproximated. The postmortem time interval between circulatory arrest and lung reperfusion was 1 hour in group 2, 2 hours in group 3, and 4 hours in group 4. Weight of the animals in all study groups is listed in Table 1.

Animal Preparation

All animals received humane care in compliance with the "Guide for the Care and Use of Laboratory Animals" published by the National Institutes of Health (NIH publication 85-23, revised 1985).

In each experiment, three rabbits were used, one as lung donor and two as additional blood donors. Animals were premedicated and anesthetized with an intramuscular injection of 0.25 ml/kg of ketamine (50 mg/ml) (Imalgène, Rhône Mérieux, Lyon, France) and 0.15 ml/kg of medetomidin chlorhydrate (1 mg/ml), paramethylhydroxybenzoate (1 mg/ml), and parapropylhydroxybenzoate (0.2 mg/ml) (Domitor, Orion Corp., Farmos, Espoo, Finland). The animals were intubated with a cannula (3.5-mm inner diameter; Mallinckrodt Medical, Athlone, Ireland) through a cervical tracheostomy, and the lungs were ventilated using a Harvard rodent ventilator model 683 (Harvard Apparatus, Inc., South Natick, MA) with room air (respiratory rate = 30 breaths/minute; tidal volume = 10 ml/kg body weight; positive end-expiratory pressure = 2 cm H_2O).

The chest was opened through a median sternotomy. Thymic tissue was excised. Pleural cavities were opened. Both superior caval veins, the inferior caval vein, the ascending aorta, and the main pulmonary artery were encircled by individual ligatures. Sodium heparin 700 IU/kg (Heparin Novo 5.000 IU/ml, Novo Nordisk, Bagsvaerd, Denmark) was administered through a marginal ear vein.

In the lung donor, the main pulmonary artery and the inferior caval vein were cannulated with a 10-gauge catheter (Angiocath, Becton Dickinson Vascular Access, Sandy, UT) and secured by a pursestring suture in the right ventricular outflow tract and the right atrial appendage, respectively. The ascending aorta was ligated and the pulmonary artery was isolated from the right ventricle by ligature around the tip of the catheter just distal to the pulmonary valve, creating pulmonary ischemia. The animal was rapidly exsanguinated through the catheter in the inferior caval vein and autologous blood was collected. All remaining ligatures were then tied. Both the endotracheal cannula and the pulmonary artery catheter remained in place until reperfusion.

Homologous blood was collected from two additional donors. The surgical procedure was as described above except that a single catheter was placed into the inferior caval vein through the right atrial appendage.

Table 2. PERFUSATE PARAMETERS DURING 4 HOURS OF REPERFUSION IN ALL EXPERIMENTS*

Parameter	Reperfusion Time (minutes)									
	5	15	30	60	90	120	150	180	210	240
Temp† (°C)	35.5 ± 0.3	35.7 ± 0.2	35.8 ± 0.2	35.9 ± 0.2	35.9 ± 0.2	35.9 ± 0.3	35.9 ± 0.3	35.7 ± 0.3	35.5 ± 0.3	35.4 ± 0.3
pH†	7.46 ± 0.01	7.47 ± 0.01	7.48 ± 0.01	7.48 ± 0.01	7.47 ± 0.01	7.46 ± 0.01	7.43 ± 0.01	7.40‡ ± 0.01	7.40§ ± 0.02	7.37‖ ± 0.02
PaO_2† (mm Hg)	48.6 ± 4.6	45.0 ± 4.6	44.8 ± 3.8	44.1 ± 3.8	42.2 ± 3.6	42.4 ± 4.2	42.5 ± 4.6	38.6‡ ± 3.7	37.0§ ± 3.6	34.7‖ ± 3.3
K† (mmol/l)	5.1 ± 0.01	5.1 ± 0.01	5.1 ± 0.01	5.2 ± 0.1	5.4§ ± 0.01	5.4‖ ± 0.01	5.5‖ ± 0.01	5.6‖ ± 0.01	5.7‖ ± 0.1	5.8‖ ± 0.01
Hb† (g/dl)	7.8 ± 0.4	7.8 ± 0.3	8.4 ± 0.2	8.4 ± 0.3	8.8‡ ± 0.3	9.1‖ ± 0.2	9.3‖ ± 0.3	9.2‖ ± 0.3	9.8‖ ± 0.3	9.9‖ ± 0.3
FP-Hb† (mg/dl)	8.7 ± 1.5	–	16.3 ± 1.5	17.9 ± 1.8	21.6 ± 1.8	26.7‡ ± 2.5	36.3‖ ± 5.6	38.1‖ ± 3.7	38.5‖ ± 3.5	45.3‖ ± 5.0

* Values are means ± standard error of mean from sixteen experiments.
† Not significant between all groups (analysis of variance with factorial analysis).
‡ $p < 0.01$ *versus* 5 min (analysis of variance with repeated measurements).
§ $p < 0.001$ *versus* 5 min (analysis of variance with repeated measurements).
‖ $p < 0.0001$ *versus* 5 min (analysis of variance with repeated measurements).
FP-Hb = free plasma hemoglobin; Hb = hemoglobin; K = potassium; PaO_2 = arterial oxygen pressure; Temp = temperature.

Preparation and Monitoring of the Perfusate

Fresh venous blood (volume 36 ± 1 ml/kg; hemoglobin 11.2 ± 0.2 g/dl) from three donor animals was collected in an empty sterile bag (with 500 ml NaCl 0.9%, Baxter, Lessines, Belgium) and stored at 4°C. Thirty minutes before reperfusion, the blood was diluted $1:4^{20}$ with modified Krebs-Henseleit bicarbonate buffer solution (composition in millimoles per liter: NaCl, 118; $NaHCO_3$, 25; KCl, 5.6; $CaCl_2$, 2.9; $MgCl_2$, 0.6; NaH_2PO_4, 1.2; and D-glucose, 11; pH 7.4, osmolarity 321 mOsm/l). Gelatin 3 g% (30 g/l) (Gelatin Type A ± 60 bloom, Sigma, St. Louis, MO) was added to this crystalloid solution as plasma expander. The reperfusion blood reservoir was then filled with the diluted blood and the circuit was primed (approximately 200 ml) and deaired. Blood and perfusate volumes in all study groups are listed in Table 1.

During the experiment, the temperature of the inflowing blood was recorded and samples were taken to monitor pH, arterial oxygen pressure, and the concentrations of potassium and blood and free plasma hemoglobin. No significant differences in these parameters were seen at any time among all groups. However, with longer reperfusion time, there was a significant decrease in pH and arterial oxygen pressure and an increase in blood hemoglobin, reflecting hemoconcentration and a rise in potassium and free plasma hemoglobin resulting from hemolysis in the extracorporeal circuit (Table 2).

Isolated Reperfusion Circuit

The closed reperfusion circuit is shown schematically in Figure 1. The heart-lung block was suspended in a humidified and temperature-controlled (37° to 38°C) plexiglas chamber from a force displacement transducer (type TB-611T, Nihon Kohden, Tokyo, Japan) by a thin rigid tube connected at the ends to the tracheal cannula and the pulmonary arterial catheter, respectively. This tube bridges the heart-lung block, thereby preventing edematous lungs from sagging during the experiment.

Pulmonary venous effluent was drained from the left atrium by gravity (60 cm H_2O) into the blood reservoir (Minimax filtered hardshell reservoir 1316, Medtronic, Anaheim, CA) submerged in a temperature-controlled (39°C) water bath. The blood was recirculated using silicone tubing and a roller pump (Model 503s, Watson Marlow, Falmouth, Cornwall, UK). The perfusate passed through a 40-μm blood filter (PALL blood transfusion filter,

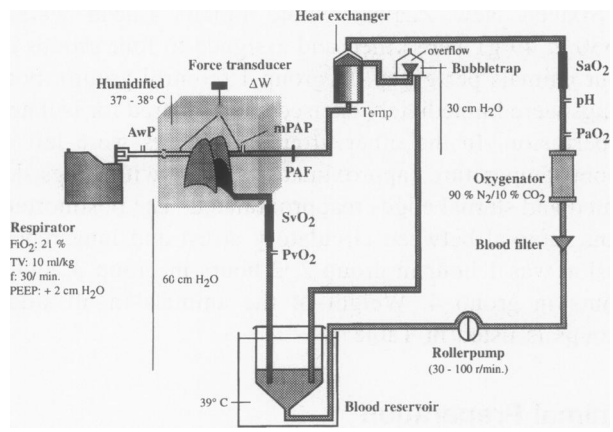

Figure 1. Schematic diagram of the isolated blood reperfusion circuit. AwP, peak airway pressure; f, frequency; Fio_2, inspired oxygen fraction; mPAP, mean pulmonary artery pressure; PAF, pulmonary artery flow; Pao_2, arterial oxygen pressure; PEEP, positive end-expiratory pressure; $P\bar{v}o_2$, venous oxygen pressure; Sao_2, arterial oxygen saturation; $S\bar{v}o_2$, venous oxygen saturation; Temp, temperature; TV, tidal volume; ΔW, weight gain.

East Hills, NY) and was then deoxygenated in a membrane gas exchanger (Minimax Hollow Fiber Oxygenator type 1381, Medtronic) using a gas mixture of 90% N_2 and 10% CO_2, obtaining partial oxygen and carbon dioxide pressures of approximately 40 mm Hg (see Table 2) and approximately 30 mm Hg, respectively. The pH of the inflowing blood was continuously monitored (see Table 2) with an in-line pH electrode (Hamilton Liq-Glass, Bonaduz, Switzerland) connected to a pH meter (WTW pH-91, Weilheim, Germany) and kept between 7.35 and 7.55 by adjusting the CO_2. Finally, the perfusate was rewarmed to approximately 36°C (see Table 2) through a heat exchanger (Cardioplegia heat exchanger D 720 Helios, Dideco, Mirandola, Italy). From there, the blood was returned to the lungs through the pulmonary artery and the perfusion pressure was kept constant at 30 cm H_2O via an overflow system (Pediatric bubble trap K-20, Quest Medical, Inc., Allen, TX). The rate of the roller pump (30 to 100 r/minute) was constantly adjusted according to the pulmonary artery flow to allow a permanent overflow via the bubble trap into the blood reservoir.

Preparation and Reperfusion of Heart-Lung Block

At the end of the ischemic period, the heart-lung block was excised from the cadaver and submerged deflated in saline solution at 37°C. A side-hole drainage catheter was inserted into the left atrium through a pursestring suture in the apex of the left ventricle. The left atriotomy was closed using a running 5-0 nonabsorbable monofilament suture. This procedure included another 28 minutes of warm ischemia and did not differ among the groups (see Table 1). Thereafter, the heart-lung block was suspended from the force transducer in the plexiglas box to measure the initial weight of the heart-lung block (see Table 1). Both pulmonary arterial and left atrial cannulas were connected to the silicone tubing and deaired.

Both lungs were then briefly reexpanded with end-expiratory pressure of more than 30 cm H_2O to remove all atelectatic zones and were further ventilated with room air (respiratory rate = 30 breaths/minute; tidal volume = 10 ml/kg body weight; positive end-expiratory pressure = 2 cm H_20) during the whole experiment. Reperfusion was started at time 0 after partial declamping of the inflowing line. The first 20 ml of outflowing blood was discarded. Thereafter, venous effluent was drained into the blood reservoir for subsequent recirculation. After a stabilization period of 10 minutes, the clamp on the inflowing line was removed completely to allow full flow.[21]

Assessment of Graft Function

The endotracheal cannula and the pulmonary artery catheter were connected to pressure transducers (Uniflow type 43-600F, Baxter, Uden, the Netherlands) and zeroed at this level to measure peak airway pressure (AwP) and mean pulmonary artery pressure (mPAP), respectively. Pulmonary artery flow (PAF) was measured with an in-line flow probe (FF-040T, Nihon Kohden) connected to an electromagnetic flow meter (MFV-1200, Nihon Kohden). The increase in weight of both lungs (ΔW) was monitored from the force transducer (see Fig. 1). During the whole experiment, AwP, mPAP, PAF, and ΔW were continuously recorded via an amplifier (Carrier amplifier AP-601G, Nihon Kohden) on a four-channel recorder (Heat writing recorder model WT-645G, Nihon Kohden). Total pulmonary vascular resistance (TPVR) was calculated using the formula (mPAP/PAF) × 1000.

Two on-line oxygen saturation probes (Bentley SMP-0110 attached to a Bentley oxysat optical transmission cell, Baxter, Irvine, CA) were connected to an oxygen saturation meter (Bentley Oxysat-meter SM-0100, Baxter) for continuous monitoring of arterial and venous oxygen saturation. Blood samples of deoxygenated inflowing (Pao_2) and oxygenated outflowing ($P\bar{v}o_2$) blood were taken for blood gas analysis (ABL 4 Radiometer A/S, Copenhagen, Denmark) at 5, 10, 15, 20, 30, 40, 50, 60, 80, 100, and 120 minutes and every 30 minutes thereafter until the end of the experiment (see Fig. 1). Venoarterial oxygen pressure gradient ($\Delta v\text{-}aPo_2$) was calculated as an estimate of the oxygenation capacity of both lungs.

Reperfusion was continued for 4 hours or until lung failure occurred (defined as PAF < 5 ml/minute). The total perfusion time was recorded as graft survival time.

No attempt was made to remove any fluid accumulated in the trachea. At the end of the experiment, both lungs were excised from the heart-lung block at the hilum, the wet weight was recorded, and the lungs were dried in an oven (model HT 600, Heraeus, Hanau, Germany) at 150°C for approximately 18 hours to constant weight. Wet-to-dry weight ratio (W/D) was calculated as an estimate of the extent of lung edema.

Statistical Analysis

Values are expressed in cm H_2O for AwP, in mm Hg for mPAP, in ml/min for PAF, in Wood units (WU) for TPVR, in percent for ΔW and graft survival, and in mm Hg for $\Delta v\text{-}aPo_2$. Data are presented as means ± standard error of the mean.

Differences within one group between values at successive time intervals of reperfusion were calculated using one-way analysis of variance with repeated measurements followed by Scheffé's multiple comparison test.[22] Differences between study groups at the same reperfusion interval were compared using analysis of variance with factorial analysis (StatView SE+ Graphics, Abacus Concepts Inc., Berkeley, CA) on a Macintosh Performa 630 computer. Values of $p < 0.05$ were accepted as significant.

Table 3. HEMODYNAMIC PARAMETERS DURING 4 HOURS OF REPERFUSION IN DIFFERENT STUDY GROUPS*

	Reperfusion Time (minutes)									
Group	**5**	**15**	**30**	**60**	**90**	**120**	**150**	**180**	**210**	**240**
mPAP†										
1: CONTR	15 ± 1	14 ± 1	12 ± 1	12 ± 1	16 ± 1	18 ± 1	18 ± 1	19 ± 1	21‡ ± 1	21† ± 1
2: DEFL-1	17 ± 1	17 ± 1	15 ± 1	15 ± 2	16 ± 2	17 ± 2	18 ± 2	19 ± 2	18 ± 1	19 ± 1
3: DEFL-2	21 ± 1	19 ± 1	17§ ± 1	17§ ± 1	18‡ ± 1	19 ± 1	20 ± 1	20 ± 0	21 ± 1	22 ± 0
4: DEFL-4	14 ± 1	14 ± 1	14 ± 1	14 ± 1	17 ± 1	18 ± 2	19 ± 2	19 ± 2	19 ± 2	21 ± 3
PAF†										
1: CONTR	66 ± 4	89 ± 2	97 ± 3	99 ± 3	75 ± 6	62 ± 9	53 ± 10	42 ± 12	30 ± 13	22† ± 12
2: DEFL-1	42 ± 12	60‖ ± 14	71‡ ± 10	64‖ ± 16	56 ± 15	48 ± 14	42 ± 14	37 ± 13	38 ± 13	31 ± 10
3: DEFL-2	8 ± 2	31‡ ± 6	49¶ ± 7	50¶ ± 5	39§ ± 10	31‡ ± 8	18 ± 6	15 ± 4	12 ± 3	6 ± 1
4: DEFL-4	54 ± 5	64 ± 5	69 ± 4	66 ± 2	44 ± 11	34 ± 13	24 ± 12	26 ± 15	30 ± 15	24 ± 13
TPVR†										
1: CONTR	227 ± 30	158 ± 7	123 ± 4	124 ± 4	215 ± 32	302 ± 48	399 ± 87	613 ± 192	1617 ± 765	8997 ± 5681
2: DEFL-1	585 ± 231	351 ± 115	228 ± 54	352 ± 166	520 ± 306	705 ± 433	890 ± 525	1136 ± 702	595 ± 235	810 ± 338
3: DEFL-2	3127 ± 592	750‖ ± 223	391‡ ± 108	354‡ ± 54	579‖ ± 171	805‖ ± 259	1619 ± 583	1595 ± 524	2214 ± 758	3632 ± 488
4: DEFL-4	266 ± 50	231 ± 27	201 ± 19	218 ± 15	516 ± 163	988 ± 421	2825 ± 1636	2246 ± 1638	867 ± 500	1289 ± 802

* Values are means ± standard error of mean from four experiments.
† Values are expressed in mmHg for mean pulmonary artery pressure (mPAP), in ml/min for pulmonary artery flow (PAF), in Wood Units (mmHg/L/min) for total pulmonary vascular resistance (TPVR).
‡ $p < 0.01$ *versus* 5 minutes by analysis of variance with repeated measurements.
§ $p < 0.001$ *versus* 5 minutes by analysis of variance with repeated measurements.
‖ $p < 0.05$ *versus* 5 minutes by analysis of variance with repeated measurements.
¶ $p < 0.0001$ *versus* 5 minutes by analysis of variance with repeated measurements.
CONTR = control; DEFL-1 = deflated 1 hour; DEFL-2 = deflated 2 hours; DEFL-4 = deflated 4 hours.

RESULTS

Hemodynamics

Hemodynamic parameters in all study groups are presented in Table 3. After the onset of reperfusion, there was a gradual decrease in both TPVR and mPAP in all deflated groups to reach a minimum after 1 hour. Concomitantly, PAF increased to reach a maximum after 1 hour. Thereafter, mPAP and TPVR increased again up to 4 hours, resulting in an important decline in PAF.

At the onset of reperfusion, however, TPVR was greater with longer warm ischemic intervals, reaching a maximum at 2 hours after death (3127 ± 592 WU in group 3 *vs.* 227 ± 30 in the control group at 5 minutes; $p < 0.001$). This was reflected by a significantly higher mPAP (21 ± 1 mm Hg *vs.* 15 ± 1 at 5 minutes, respectively; $p < 0.01$) and resulted in a significantly lower PAF (8 ± 2 ml/min *vs.* 66 ± 4 at 5 minutes, respectively; $p < 0.001$). These differences in TPVR, mPAP, and PAF among the groups disappeared after 1 hour of reperfusion.

Graft survival time during reperfusion also decreased with longer periods of warm ischemia. Survival at 210 minutes of reperfusion was 100% in the control group, 75% in group 2, 75% in group 3, and 50% in group 4 (Table 4).

Aerodynamics

The evolution in AwP during reperfusion in all groups is depicted in Figure 2. After the onset of reperfusion, there was an increase in AwP with longer warm ischemic inter-

Table 4. GRAFT SURVIVAL* DURING 4 HOURS OF REPERFUSION IN DIFFERENT STUDY GROUPS

	Reperfusion Time (minutes)									
Group	**5**	**15**	**30**	**60**	**90**	**120**	**150**	**180**	**210**	**240**
1: CONTR	100	100	100	100	100	100	100	100	100	50
2: DEFL-1	100	100	100	100	100	100	100	100	75	75
3: DEFL-2	100	100	100	100	100	100	100	75	75	75
4: DEFL-4	100	100	100	100	100	100	75	75	50	50

* Percentages of lungs (n = 4) with pulmonary artery flow >5 ml/min.
CONTR = control; DEFL-1 = deflated 1 hour; DEFL-2 = deflated 2 hours; DEFL-4 = deflated 4 hours.

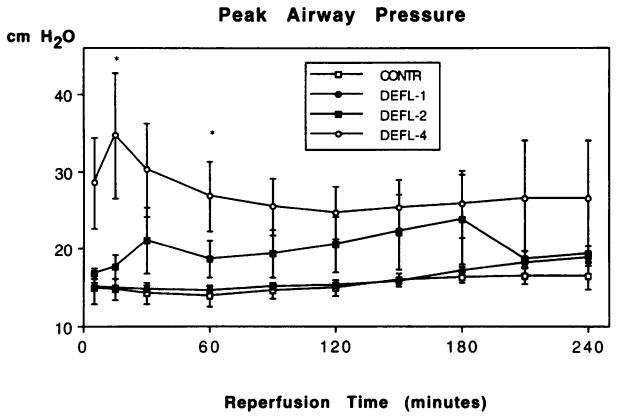

Figure 2. Peak airway pressure during 4 hours of isolated reperfusion, comparing control lungs that were immediately reperfused (CONTR) with lungs left deflated at room temperature (24°C) for 1 hour (DEFL-1), 2 hours (DEFL-2), and 4 hours (DEFL-4) after death. Mean values (±SEM) from four experiments are presented. ($^{*}p < 0.05$ for DEFL-4 *vs.* CONTR by analysis of variance with factorial analysis).

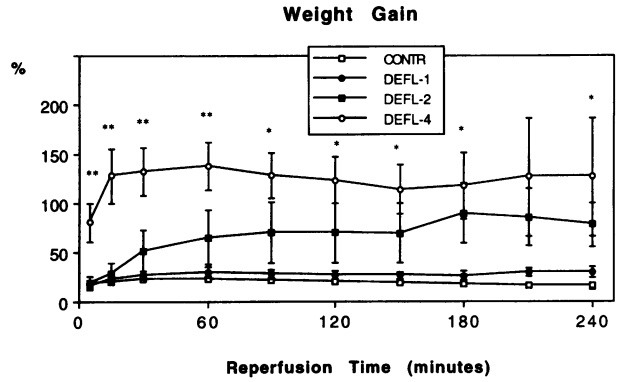

Figure 4. Weight gain during 4 hours of isolated reperfusion, comparing control lungs that were immediately reperfused (CONTR) with lungs left deflated at room temperature (24°C) for 1 hour (DEFL-1), 2 hours (DEFL-2), and 4 hours (DEFL-4) after death. Mean values (±SEM) from four experiments are presented. ($^{*}p < 0.05$, $^{**}p < 0.01$ for DEFL-4 *vs.* CONTR by analysis of variance with factorial analysis).

vals, reflecting a decrease in static lung compliance as a result of edema formation. AwP in group 4 was significantly greater than in the control group (34.7 ± 8.1 cm H_2O *vs.* 14.8 ± 1.3 at 15 minutes; $p < 0.05$). No difference in AwP was observed at any time interval between the control group and group 2.

Oxygenation Capacity

The difference in partial oxygen pressure between deoxygenated inflowing and oxygenated outflowing blood is depicted in Figure 3. There was a decline in oxygenation capacity with longer warm ischemic intervals (14 ± 6 mm Hg in group 4 and 47 ± 15 in group 3 *vs.* 122 ± 10 in the control group at 30 minutes; $p < 0.0001$ and < 0.01, respectively). No significant difference in $\Delta v\text{-}aPo_2$ was seen at any time interval between the control group and group 2.

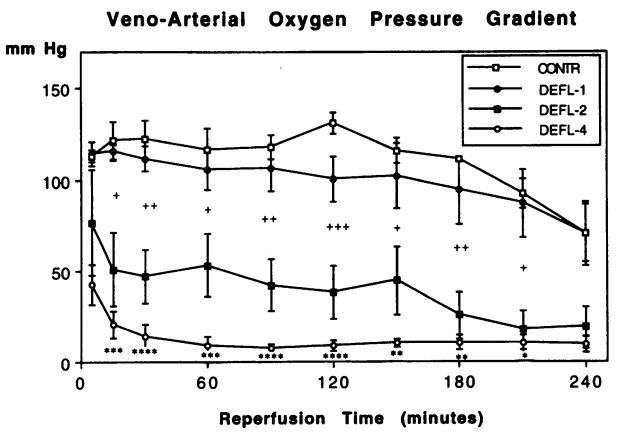

Figure 3. Venoarterial oxygen pressure gradient during 4 hours of isolated reperfusion, comparing control lungs that were immediately reperfused (CONTR) with lungs left deflated at room temperature (24°C) for 1 hour (DEFL-1), 2 hours (DEFL-2), and 4 hours (DEFL-4) after death. Mean values (±SEM) from four experiments are presented. ($^{+}p < 0.05$, $^{++}p < 0.01$, $^{+++}p < 0.001$ for DEFL-2 vs. CONTR; $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$, $^{****}p < 0.0001$ for DEFL-4 *vs.* CONTR by analysis of variance with factorial analysis).

Edema Formation

The increase in weight during reperfusion is depicted in Figure 4. In nonischemic lungs (the control group), the weight of the heart-lung block increased by 18 ± 1% at 5 minutes after the onset of reperfusion. This ΔW was the result of vascular distention by an increased blood volume, and not from filtration of fluid out of the microvasculature into the lung interstitium. The increase in weight, however, augmented with longer ischemic intervals (137 ± 24% in group 4 *vs.* 23 ± 1% in the control group at 1 hour; $p < 0.01$). No difference in ΔW was observed at any time interval between the control group and group 2.

Finally, W/D at the end of reperfusion in all groups is depicted in Figure 5. There was an important increase in W/D with longer warm ischemic intervals (5.4 ± 0.3 in the control group, 5.8 ± 0.1 in group 2, 8.1 ± 1.6 in group 3, and 10.2 ± 1.0 in group 4; $p < 0.05$ group 4 *vs.* the control group).

DISCUSSION

In this isolated reperfusion study, we have demonstrated that pulmonary graft function after a period of warm ischemia is time-dependent. Lung function started to deteriorate after 2 hours of warm ischemia. Severe reperfusion injury with significantly reduced oxygenation, elevated peak airway pressure suggesting impaired lung compliance, and significant weight gain occurred after a 4-hour period of circulatory arrest. No significant differences in graft function, however, were observed in lungs subjected to 1 hour of warm ischemia when compared with nonischemic lungs.

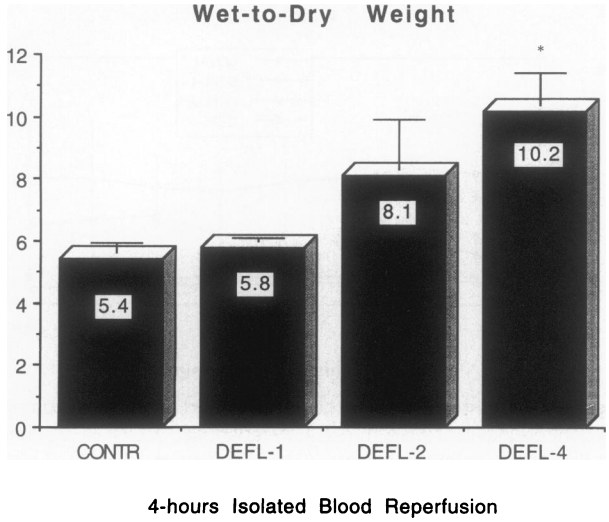

Figure 5. Wet-to-dry weight ratio of pulmonary grafts that were reperfused up to 4 hours, comparing control lungs that were immediately reperfused (CONTR) with lungs left deflated at room temperature (24°C) for 1 hour (DEFL-1), 2 hours (DEFL-2), and 4 hours (DEFL-4) after death. Mean values (±SEM) from four experiments are presented. (*$p < 0.05$ for DEFL-4 *vs.* CONTR by analysis of variance with factorial analysis).

This suggests that the warm ischemic tolerance of a collapsed lung is limited to approximately 60 minutes.

This study validates the conclusions of a previous functional study from our laboratory in which cadaveric rabbit lungs were flushed with a cold crystalloid solution at increasing time intervals after death.[17] Pulmonary edema developed in atelectatic lungs when hypothermic flush was delayed for 2 hours after death. Graft function, assessed by measuring AwP during flush and W/D at the end of the flush, however, was not different in lungs after a delay of up to 1 hour after death when compared with nonischemic, control lungs flushed in heart-beating animals. From that study, we already concluded that the warm ischemic tolerance in the nonexpanded lung is limited to 60 minutes. These flush experiments were used as a rapid screening method to define the length of tolerable warm ischemia before we started to use our isolated reperfusion model to assess graft function, including oxygenation capacity.

In another study from our laboratory using this isolated rabbit lung reperfusion model, we have demonstrated that this limited interval of 1 hour could be extended up to 4 hours after death by inflating or ventilating the lungs inside the warm cadaver.[18] The mechanism of this protective effect appeared to be independent of the method of lung stretching (static or intermittent) and was not influenced by the gas mixture (room air or nitrogen) used for alveolar ventilation. From that study, we concluded that prevention of alveolar collapse in the NHBD was the critical factor to protect the lung from warm ischemic damage independent of continuous oxygen supply. This finding is in contrast with the observations made by Weder et al.,[23] who examined the role of intraalveolar oxygen concentration on lung preservation during cold ischemia. They reported superior gas exchange in isolated rabbit lungs that were reperfused with deoxygenated blood after 24 hours of hypothermic storage when the donor lungs were inflated with 100% oxygen as opposed to room air. Lungs that were inflated with 100% nitrogen rapidly developed pulmonary edema and could not be reperfused for 60 minutes.

Many historical lung preservation studies before the use of hypothermic flush and storage have already demonstrated that collapse of the lung during interruption of circulation should be avoided. In 1953, Blades et al.[24] reported considerable impairment of gas exchange in dogs dependent on lungs subjected to more than 60 minutes of *in situ* normothermic ischemia. In an isolated, perfused canine lung model, Homatas et al.[25] in 1968 demonstrated that ventilating the lungs with room air within the cadaver permitted a safe holding period of 4 to 6 hours *versus* only 2 hours in nonventilated cadavers. The effect of ventilation and inflation on warm ischemic tolerance of the lung was also investigated by Veith et al.[26] in 1971 in a canine unilateral transplant model. The animals survived totally dependent on the transplanted lung after 30 minutes of ischemia with the lung collapsed, after 2 hours of ischemia with the lung ventilated, and after 3 hours when the lung was inflated. The beneficial effect of preventing the lung from collapsing below functional residual capacity during normothermic ischemia was stressed in several canine studies in the mid-1970s by authors from the UCLA School of Medicine.[27–29]

More recently, the University of North Carolina group at Chapel Hill demonstrated the feasibility of transplanting lungs retrieved at intervals after death and subjected to an additional period of cold storage after hypothermic Euro-Collins flush. Canine lungs retrieved 1 hour after death showed excellent gas exchange after transplantation. Lungs extracted 2 hours after death showed inconsistent gas exchange, and lungs retrieved 4 hours after death demonstrated poor oxygenation in the allograft recipient.[7] However, gas exchange in lungs harvested 4 hours after death was substantially improved if the donor had been ventilated after cessation of circulation.[8]

After the onset of reperfusion, the pulmonary arterial flow was progressively reduced with longer postmortem ischemic intervals up to 2 hours. At 4 hours after death, the pulmonary vascular resistance was as low as in lungs with a 1-hour ischemic interval but was still greater than in control lungs. It is well known that the vasculature of an ischemic organ becomes more difficult to perfuse (so-called no-reflow phenomenon), and as a consequence that the reperfusion injury becomes more apparent as the duration of ischemia is prolonged.[30] The exact mechanism remains unclear. Hypoxic vasoconstriction, mediator-induced vasospasm, endothelial cell swelling, and microvascular plugging of cellular blood elements have all been recognized as possible causes of increased resistance.

Warm ischemia in lungs will rapidly disturb pulmonary metabolism. We have found that the concentration of mitochondrial high-energy ATP reserves in deflated lungs

dropped to nearly 25% of the preischemic level at 30 minutes after circulatory arrest.[13] As a result, pulmonary cells lose their maintenance of selective membrane permeability, resulting in the release of lytic enzymes normally sequestered in the lysosomes. Using a trypan blue vital dye exclusion test, we have demonstrated that there was a 3.2-fold increase in the number of nonviable pulmonary cells at 30 minutes after circulatory arrest.[14] Postischemic endothelial cell damage may then lead to permeability pulmonary edema and clinically apparent acute graft dysfunction after reperfusion.[31]

For many years, investigators have shown interest in the use of extrathoracic organs from NHBDs.[5] The main question regarding the use of these donors revolves around the issue of tolerance to warm ischemia. How much warm ischemia can a specific organ tolerate and still be expected to yield acceptable results after transplantation? Centers with experience in the use of organs from "controlled"[19] NHBDs consider up to 2 hours of warm ischemic time as acceptable for kidneys and up to 1 hour for livers.[5] Recently, experimental work has shown that lamb hearts[32] and baboon hearts[33] could be transplanted and resuscitated after as much as 30 minutes of asphyxia, and dog hearts even after 60 minutes.[34]

Our isolated reperfusion model is simple and inexpensive and proved useful as a rapid screening method to look for factors that may improve the quality of the preserved lung allograft. It is, however, limited by the short period of graft assessment and by the side effects of prolonged extracorporeal circulation. The findings from this study, therefore, should be confirmed in a large animal model assessing survival. We plan to investigate the use of lungs from NHBDs in a canine allotransplant survival model, as previously described.[7]

In summary, warm ischemia results in increased pulmonary vascular resistance. Graft function, assessed by monitoring peak airway pressure, oxygenation capacity, and edema formation during reperfusion of deflated lungs retrieved up to 1 hour after death, was not significantly worse than in nonischemic lungs. From this study, we therefore conclude that 60 minutes of warm ischemia in the collapsed lung may be tolerated before cold storage. Further studies are necessary to investigate whether lungs from human NHBDs will become a realistic alternative to expand the pulmonary donor pool.

Acknowledgments

The authors thank Peter Lemmens, Magda Mathys, Eddy Vandezande, Gerda Ceulenaere, and Kanigula Mubagwa, MD, PhD, for expert technical and secretarial assistance.

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